NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 34, 2017
Print ISSN 1222-4227; Online ISSN 2067-5720

EFFECTS OF MAIZE EXPRESSING THE INSECTICIDAL PROTEIN CRY1AB ON NON-TARGET GROUND BEETLE ASSEMBLAGES (COLEOPTERA, CARABIDAE)

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ABSTRACT

Ground beetle fauna, as well-known worldwide bioindicators, were studied in the large-scale Bt maize experiment in Poland. The aim of this study was to determine the impact of the Bt maize variety in comparison to conventional plants on species from ground beetle assemblages (Coleoptera: Carabidae) as non-target arthropods. A genetically modified Bt maize variety (DKC 3421 Yield Gard®, event MON 810) and its near-isogenic DKC 3420 (unsprayed and sprayed with a lambda-cyhalotrine insecticide) were cultivated at 2 locations: Budziszów, near Wrocław in south-western Poland, and in Głuchów, near Rzeszów in the south-eastern region, in 2008-2010. For comparative analysis two additional non-Bt varieties (Bosman and Wigo) were also included. To monitor population density of ground beetles, 80 pitfall traps were used at each location. Comparisons showed no significant differences between abundance and diversity patterns of ground beetles for the Bt maize, the near isoline and two local varieties. Some minor differences were with no clear tendencies related to Bt maize.

Key words: bioindicators, Bt maize, epigeal arthropods, environmental risk, transgenic crops.

INTRODUCTION

Adoption of transgenic crops should always be supported by widespread assessment of ecological risk with potential adverse effects on non-target arthropods affecting the biodiversity of agro-ecosystems (Hilback et al., 2009; Hui-Lin et al., 2011; Naranjo, 2009). The gene expressing delta-endotoxin Cry 1Ab from *Bacillus thuringensis* Berliner var. *kurstaki* is usually used only against well-defined caterpillars from lepidopteran order that feed on maize. In Europe, the most important pest species are the European corn borer (*Ostrinia nubilalis* Hübner, Crambidae) and, in the Mediterranean Basin, pink stem borer (*Sesamia nonagrioides* Lefëbvre, Noctuidae) (Meissle et al., 2014). One of the strategies to control maize borers is to cultivate genetically modified plants (GM). Bt maize expressing the insecticidal protein Cry1Ab very effectively controls the primary targets, as *Ostrinia nubilalis* (Bereś, 2010). In plant protection this prevents economic loss throughout the season, consequently eliminating the requirement for extensive monitoring and insecticide application against caterpillars. Bt maize has been grown commercially since 1996 (United States), and from this time the area under such GM varieties rapidly increased worldwide (Meissle et al., 2009).

Ground beetles are one of the key organisms in maize field ecosystem, often acting as generalist predators of many crop pests (Holland, 2002; Lang et al., 1999; Lopez et al., 2005). These soil-dwelling insects are exposed to Bt proteins directly through ingesting maize litter and pollen, and indirectly through eating prey that feeds on Bt maize plants. They can also act as natural enemies of pests feeding on maize, which is another possible way of being in contact with the GM-plant material ingested by the herbivores. In the soil ecosystem, the detection of the ecological

Received January 2016; accepted 7 February 2017. First online: March, 2017. DII 2067-5720 RAR 2017-9

consequences of exposure to Bt maize is difficult because sampling is time-consuming and there is high variability among samples. The ecological requirements of Carabidae are known and well defined for many species that form assemblages in cultivated fields, making them excellent bioindicators (Koivula, 2011). Moreover, some species can be herbivorous, detrivorous or carnivorous, important in controlling maize pests. Such species can be appropriate for describing the trophic relationships and degree of biodiversity in the soil. This coleopteran group is also thought to be an important component of both natural and agricultural ecosystems, contributing to the regulation of prey species and biological control of pests, and even having a role in the improvement of physical soil parameters (Symondson et al., 2002; Thiele, 1977). Ground beetles' contribution to biodiversity, as they are a species rich arthropod family, also results from the relatively high biomass and usually high number of species collected in a particular area. Thus, soil-dwelling Carabidae have been identified as good candidates for evaluating the potential unintended effects of transgenic crops (Lopez et al., 2005).

The aim of the study was to determine the impact of the Bt maize variety MON 810, expressing the Cry1Ab protein, aimed at controlling lepidopteran pests, on abundance and species composition of non-target ground beetles (Coleoptera: Carabidae) in comparison to conventional maize varieties. Evaluations of the effects of Bt toxin on Carabidae have been performed as part of broader field studies aimed at determining the effects of GM plants on non-target arthropods within the maize ecosystem.

MATERIAL AND METHODS

Site properties and study design

The direct environmental effects of the Bt gene were tested through the studies conducted in maize fields at 2 locations in the southern part of Poland (central Europe), i.e. in Budziszów (51°06' N, 17°02' E), near Wrocław, and in Głuchów (50°01″ N, 22°17″ E), near Rzeszów (distance between locations about 400 km), in the three sampling periods from 2008 to 2010. A large-scale experiment was allocated in the region where the majority of maize is cultivated in Poland, and where infestation by the European corn borer is a substantial problem (Bereś, 2010). The following treatments were used in the experiment: (1) Bt transgenic maize (DKC 3421 Yield Gard®, event MON 810), (2) its near-isogenic non-Bt variety without insecticide application (DKC 3420), (3) isogenic non-Bt variety (DKC 3420) with lambda-cyhalotrine treatment, and for comparative analysis 2 non-Bt conventional varieties (4) Bosman and (5) Wigo sprayed with lambda-cyhalotrine were also included (as reference control Ref. 1 and Ref. 2, respectively). The reference varieties had similar FAO numbers but were otherwise unrelated to the MON 810 and DKC groups. Each year the insecticide (active ingredient: lambda-cyhalotrine in Karate Zeon 050 CS) was applied once in selected treatments in the 2nd half of July at maize stage BBCH 55-59, in a dose of 0.2 l/ha. Weed control was done with nikosulfuron (Milagro Extra 6 OD), shortly after maize emergence (in the 2 half of May), on the entire area of the experiment. This herbicide has low potential for bioaccumulation, and is not persistent in soil. Neither fungicides nor other pesticides were applied. All the agro-technology applied in the maize field, including fertilizers, was identical on the entire area of the experiment. The design of this experiment consisted of randomised complete blocks with 5 treatments and 4 replications. For the experimental design a large size plot was set up (40 x 40 m). An alley distance of 4.5 m was used between 1600 m^2 plots. Experiments were conducted on the same plots for 3 consecutive years (in both locations), hence the growing amount of Bt endotoxin we could expect on the same plots. Detailed design and description of the field experiment was included in Twardowski et al. (2014).

Ground beetle collection

A total of 80 circular pitfall traps were set up in each location to collect the epigeal arthropods. 4 traps were arranged on each plot, and the distance between them was at least 10 m, with a 10 m distance from plot margins. The arrangement of the traps within plots was performed so as to avoid any side-effects. These were inserted into the ground so that the lip was flush with the soil surface. Each trap was a plastic container with a 9 cm diameter and 12 cm deep, filled with 50:50 water and ethylene glycol used as a preservative, and was sunk into another plastic tube. A square plastic, transparent lid protected the trap against precipitation. The traps were emptied weekly from the beginning of June (plants with 4-6 leaves) until maize maturation (the 2 half of September). In this period, traps were checked in 7-day intervals and emptied in the sample container, and later ground beetles were separated from the other invertebrates. In each of the 3 growing seasons 14-16 sample sets were collected (datasets). All Carabidae were identified to the species level, and nomenclature was taken from Hůrka key (1996). Only adult specimens were considered for analysis.

Data analysis

The differences between ground beetles assemblages collected from each maize varieties through whole of each sampling period was checked by ANOVA variance analyses with repeated measures and consequently Tukey's honest significant difference test (post-hoc). The significant level of $p \leq 0.05$ was considered for all tests. For this statistical analysis Statistica version 10.0 was chosen. To avoid seasonal trends influence, statistical analyses were calculated separately for each date. To compare the diversity aspects of ground beetle assemblages and to assess their similarities, the following ecological indices were applied: Shannon index ($H' = -\Sigma\ pi \ln(pi)$, where pi = the decimal fraction of individuals belonging to the ith species) with the evenness index ($E = H'/\ln S$, where H' is the Shannon index and S is the number of species). So, to compare the seasonal magnitudes of DKC 3421 YG to other/secondary diversities, we used the t-test of Hutcheson for Shannon measures: $t = |H'1 - H'2| / \sqrt{(Var + Var\ H'1H'2)}$ with $Var(H') = (\Sigma\ pi \ln(pi) - \Sigma(pi \ln(pi))^2) / N + ((S - 1) / (2N)^2)$, where: N = total number of individuals, S = number of species collected. The structure of the ground beetles community was analysed with the use of detrended correspondence analysis (DCA), without down-weight of rare species and rescaling of the axis. Prior to analysis, species abundance data were log10 (x+1) transformed. The software CANOCO, version 4.5, was performed for this analysis.

RESULTS AND DISCUSSION

Quantitative and qualitative differences in ground beetle assemblages

During three years of our study, a huge number of ground beetles were collected in 14-16 data-sets/year. More than 179,000 beetles were collected in Budziszów, and more than 127,000 in Głuchów (Table 1). There were very few significant differences in non-target ground beetle populations resulting from this experiment. All of them were found in one locality, i.e. Budziszów. Both in the first year of the study (2008) as well as in the second (2009), the abundance of studied insects collected throughout the whole growth season was significantly higher on reference variety Bosman in comparison to isogenic maize line DKC 3420 (non-protected with lambda-cyhalotrine). Only in the third year of the research (2010) was the whole assemblage of beetles found on conventional variety Bosman significantly greater than on transgenic variety DKC 3421 YG. No differences were calculated in relation to 3 other compared treatments. In the second locality, i.e. Głuchów, analysis did not demonstrated any significant changes when individuals caught during whole sampling period in particular treatments were compared. Despite considerable quantitative differences between locations, the range of proportions in the number of the beetles caught between treatments at each location showed considerable stability from year to year. The variation in the number of caught beetles in the period of 3 years for DKC 3421 YG and the isogenic non-protected variety DKC 3420 only reached 3%.

Table 1. Total number and significance of differences in ground beetles collected on each treatment in 2008-2010

Treatment	2008	2009	2010	Total
	Budziszów			
Ref. 1 (Bosman) Prot.	25292 a*	9294 a	11599 a	46185
Ref. 2 (Wigo) Prot.	22470 ab	7349 ab	10380 ab	40199
DKC 3420 Prot.	17340 ab	7855 ab	9162 ab	34357
DKC 3420 Non-Prot.	14640 b	5368 b	7641 ab	27649
DKC 3421 YG	17085 ab	6688 ab	6923 b	30696
Total number	96827	36554	45705	179086
	Głuchów			
Ref. 1 (Bosman) Prot.	10702	6367	7846	24915
Ref. 2 (Wigo) Prot.	11082	6776	6780	24638
DKC 3420 Prot.	10009	7167	8340	25516
DKC 3420 Non-Prot.	9999	7372	8005	25376
DKC 3421 YG	11190	7096	8282	26568
Total number	52982	34778	39253	127013

*Different letters in the columns denote significant differences between treatments (separately for each year and location).

The majority of ground beetle assemblage studies conducted in other countries show no significant differences between Bt and isogenic maize (de la Poza et al. 2005; Farinos et al., 2008; Leslie et al., 2007; Lopez et al., 2005; Priestley and Brownbridge, 2009; Szekeres et al., 2006). In a 6-year monitoring of non-target organisms in Germany, Schorling and Freiser (2006) did not find general tendencies in differences in the population densities of Carabidae, as well as of other arthropods. Within performed experiment no clear differences were also detected in case of other epigeal group, i.e. rove beetles (Twardowski et al., 2014). A few reports suggested some minor differences. A change in the ground beetle community structure was found by Toschki et al. (2007), but only in the first year of their 3-year study. These authors concluded that a more unfavourable microclimate and massive European corn borer infestation caused the differences rather than the direct toxic effects linked with Bt maize.

In all the years of the study and in two localities, 1 or 2 predominant species were found representing more than half of all collected individuals (Tables 2a and 2b). In Budziszów, in each year of the study, omnivorous *Pseudoophonus rufipes* was definitely the most abundant species (Table 2a). This species is common and usually very frequent in arable fields in Central Europe, especially on lighter soils (Thiele, 1977). In 2008, in the above-mentioned locality, 50 species were identified altogether. *P. rufipes* was most numerous on every treatment of the experiment. Besides them, the abundance of other species did not exceed 6%. Other numerous species on the 5 analysed treatments were: *Calathus fuscipes*, *Calathus erratus, Pterostichus melanarius* and *Dolichus halensis.* They are known as relatively more predatory ground beetles, consuming important pest species (Sunderland, 2002). In 2009, the dominance level of *P. rufipes* was slightly lower than in the first year. On every maize treatment the abundance of this species was topmost, from 43.7% in Ref. 2 to 50.6% in isoline maize variety DKC 3420 (non-protected). Other predominant species were *D. halensis* and *P. melanarius* (their number clearly exceeded 10% of ground beetle assemblages). The remaining 46 identified species in this year were not more numerous than 7.1% of all recorded Carabidae (DKC 3420 maize variety, non-protected). The described situation was quite similar in every treatment of the experiment, and in the last year of the field study in Budziszów, *P. rufipes* was still noted in very high numbers on every treatment (from 68.4% in Bt maize to 75% in isogenic line). The remaining 4 species' densities did not exceed 10% (with one exception, *P. melanarius*, in Bt stand 10.1%). This 10% threshold was also not exceeded by 50 other species identified in the whole experiment.

In the second locality, i.e. Głuchów, *P. melanarius* was clearly the dominant species (Table 2b). In the first year (2008) its abundance comprised from 46.1 (DKC 3420 Prot.) to 51.9% (Ref. 1). Much less abundant, but still as dominant was *P. rufipes* (34.5% - Ref. 1 to 39.5% - DKC 3420 Prot.). Besides them, the abundance of other species did not exceed 5%. There were also 3 other numerous species: *Bembidion quadrimaculatum, D. halensis* and *B. femoratum*. A similar situation, with dominant species, was almost repeated in the next 2 years. In the first year, in the above-mentioned locality, the highest number of identified species was noted (68 at all the treatments). In 2009 the dominance level of *P. melanarius* was emphatically higher than that in the first year. On every maize treatment the abundance of this species was again topmost, from 80.9% in the non-protected isogenic line to 83% in DKC 3421 YG. Visibly less frequent than in the previous year was the second species on that list, i.e. *P. rufipes* (4.2% - non-protected DKC 3420 to 6.5% - Ref. 1). The remaining species did not exceed 6.3% of all collected beetles (Ref. 1). Altogether 55 species were identified in the above-mentioned year. In the last year of the study in Głuchów (2010), the dominant *P. melanarius* comprised from 81.4% (Ref. 2) to 85.8% in Bt maize stand. Second, as regards the number, was again *P. rufipes* in similar abundance as in previous year, i.e. 5.9% in DKC 3421 YG to 8.8 in the reference variety Wigo. The remaining 42 species identified in this year were not more numerous than 4.7% of all recorded Carabidae (in Ref. 1). In this year the lowest number of species was noted altogether (47).

Table 2. Abundance of the 5 most numerous ground beetle species recorded on 5 treatments of the experiment at 2 localities in 2008-2010

Year	Species	Ref. 1		Ref. 2		DKC 3420 Prot.		DKC 3420 Non-Prot.		DKC 3421 YG		Total
		No.	%	No.	%	No.	%	No.	%	No.	%	
a) Budziszów												
2008	*Pseudoophonus rufipes*	20857	82.5	13978	80.6	17931	79.8	11985	81.9	13804	80.8	78555
	Calathus fuscipes	1080	4.3	925	5.3	1040	4.6	707	4.8	879	5.1	4631
	Calathus erratus	973	3.8	873	5.1	1335	5.9	590	4.1	712	4.2	4483
	Pterostichus melanarius	822	3.3	857	5.0	962	4.3	710	4.8	967	5.7	4318
	Dolichus halensis	1140	4.5	264	1.5	745	3.3	314	2.1	379	2.2	2842
	Remaining 45 species	420	1.6	443	2.5	457	2.1	334	2.3	344	2.0	1998
2009	*Pseudoophonus rufipes*	4302	46.3	3978	50.6	3213	43.7	2463	45.9	3023	45.2	16979
	Dolichus halensis	2455	26.4	1299	16.5	1813	24.7	990	18.4	1263	18.9	7820
	Pterostichus melanarius	1635	17.6	1605	20.4	1409	19.2	1342	25.0	1675	25.0	7666
	Calathus fuscipes	220	2.4	229	2.9	226	3.0	132	2.5	191	2.9	998
	Calathus erratus	104	1.2	146	1.9	202	2.7	83	1.5	111	1.7	646
	Remaining 46 species	578	6.1	598	7.1	486	6.7	358	6.7	425	6.3	2445
2010	*Pseudoophonus rufipes*	8344	72.0	5676	74.3	7494	72.2	6867	75.0	4736	68.4	33117
	Pterostichus melanarius	586	5.1	592	7.6	514	4.9	657	7.2	701	10.1	3050
	Anchomenus dorsalis	699	6.0	389	5.1	714	6.9	449	4.9	526	7.6	2777
	Dolichus halensis	781	6.7	153	2.1	555	5.3	239	2.6	130	1.9	1858
	Anisodactylus binotatus	236	2.0	258	3.5	311	3.0	206	2.2	168	2.4	1179
	Remaining 50 species	953	8.2	573	7.5	792	7.7	744	8.1	662	9.6	45705

Table 2 continued

b) Głuchów												
2008	*Pterostichus melanarius*	5556	51.9	5522	49.8	5056	50.6	4612	46.1	5458	48.8	26204
	Pseudoophonus rufipes	3687	34.5	3875	35.0	3610	36.1	3951	39.5	4192	37.5	19315
	Bembidion quadrimaculatum	439	4.1	563	5.0	391	3.9	480	4.8	540	4.8	2413
	Dolichus halensis	429	4.0	442	4.0	286	2.9	273	2.7	347	3.1	1777
	Bembidion femoratum	101	0.9	141	1.3	125	1.3	111	1.1	105	0.9	583
	Remaining 63 species	490	4.6	539	4.8	531	5.3	582	5.8	548	4.9	2690
2009	*Pterostichus melanarius*	5075	81.0	5660	83.5	6130	83.2	5802	80.9	5887	83.0	28554
	Pseudoophonus rufipes	412	6.5	337	5.0	308	4.2	415	5.8	356	5.0	1828
	Bembidion quadrimaculatum	168	2.6	197	2.9	332	4.5	243	3.4	206	2.9	1146
	Bembidion femoratum	223	3.5	182	2.7	207	2.8	258	3.6	262	3.7	1132
	Dolichus halensis	87	1.4	91	1.3	87	1.2	86	1.2	63	0.9	414
	Remaining 50 species	402	6.3	309	4.6	308	4.2	363	5.1	322	4.5	1704
2010	*Pterostichus melanarius*	6524	83.2	5517	81.4	6581	82.2	6948	83.3	7109	85.8	32679
	Pseudoophonus rufipes	515	6.6	599	8.8	621	7.8	693	8.3	492	5.9	2920
	Agonum mulleri	271	3.5	255	3.7	247	3.1	223	2.7	229	2.8	1225
	Poecilus cupreus	90	1.1	84	1.2	116	1.4	120	1.4	85	1.0	495
	Bembidion quadrimaculatum	76	1.0	86	1.2	88	1.1	78	0.9	69	0.8	397
	Remaining 42 species	370	4.7	239	3.5	352	4.4	278	3.3	298	3.6	1537

Only species represented by at least 10 individuals are listed in the table. The following species were caught in lower numbers in random mode throughout the experimental field, and are included in the remaining species counts at the bottom of the table:

1) only in Budziszów: *Amara apricaria, A. aulica, A. communis, A. consularis, A. ovata, Agonum marginatum, A. versutum, A. viduum, Carabus coriaceus, Cychrus caraboides, Chlaenius tristis, Harpalus calceatus, H. rubribes, Notiophilus aquaticus, Ophonus azureus.*

2) only in Głuchów: *Amara familiaris A. fulva, Agonum sexpunctatum, Badister bullatus, Brachinus crepitans, Carabus cancellatus, C. ullrichi, Chlaenius nitidulus, Drypta dentata, Dyschirius globosus, D. intermedius, Lasiotrechus discus, Oodes helopioides.*

3) species found in both localities: *Amara eurynota, A. aenea, A. bifrons, A. similata, A. plebeja, Asaphidion flavipes, Acupalpus meridianus, Broscus cephalotes, Bembidion lampros, B. obtusum, B. properans, Calathus ambiguus, C. melanocephalus.*

C. micopterus, C. hortensis, C. granulatus, Clivina fossor, Cicindela germanica, Chlaenius nigricoris, Harpalus affinis, H. distinguendus, H. latus, H. tardus, H. smaragdinus, Leistus ferrugineus, Loricera pilicornis, Microlestes minutulus, Nebria brevicollis, Ophonus brevicollis, Platynus assimilis, Poecilus lepidus, P. versicolor, Pterostichus niger, P. nigrita, P. strenuus, P. oblongopunctatus, P. vernalis, Pseudoophonus griseus, Stomis pumicatus, Zabrus tenebrioides.

Ecological analysis of species composition

The diversity of captured species is shown in Table 3 (Budziszów) and Table 4 (Głuchów). Generally, the diversity values calculated for the first site in the 3 years of the study were higher compared to the second location. In Budziszów, throughout all the research seasons clear differences between both reference and DKC varieties were visible (Table 3). In the first 2 years, within the DKC group no statistical differences were found. In the year 2010, the Shannon index value within the entire experiment was average, although on plots of ground DKC 3421 YG was substantially highest, at the highest sample evenness (E=0.37). On the other hand, the lower number of captured individuals may also have had an influence on the index value. Described later detrended correspondence analysis did not confirm these significant differences between examined treatments found at that site (Figure 1).

In the second locality, in Głuchów (2008), a significantly higher value of Shannon index was found on the DKC unprotected variety, but the following year the opposite was found on protected DKC and Ref. 1 varieties (Table 4). In both seasons for these cases, we found differences between the value of their evenness (E) or numbers of captured specimens. The strength of the dominant position within species structure also influences the index value. In 2010, in DKC 3421 YG, the strong domination of one species was found.

Table 3. Ecological analysis of ground beetle diversity in Budziszów in 2008-2010

Diversity parameters	Ref. 1 Prot.	Ref. 2 Prot.	DKC 420 Prot.	DKC 3420 Non-Prot.	DKC 3421 YG
2008					
No. of individuals (N)	25292	22470	17340	14640	17085
No. of species (S)	37	36	34	31	38
Shannon Index (H`)	0.78	0.87	0.85	0.81	0.83
H/log(N) evenness of samples	0.22	0.24	0.24	0.24	0.23
Var H`	0.00007	0.00008	0.00011	0.00013	0.00011
t-test* difference DKC 3421 YG to:	3.89	2.61	ns	ns	
2009					
No. of individuals (N)	9294	7349	7855	5368	6688
No. of species (S)	39	32	40	38	33
Shannon Index (H`)	1.48	1.57	1.54	1.53	1.52
H/log(N) evenness of samples	0.4	0.45	0.42	0.42	0.44
Var H`	0.00016	0.00019	0.00022	0.00028	0.00021
t-test* difference DKC 3421 YG to:	2.14	2.34	ns	ns	
2010					
No. of individuals (N)	11599	10380	9162	7641	6923
No. of species (S)	35	36	37	34	33
Shannon Index (H`)	1.18	1.2	1.11	1.13	1.28
H/log(N) evenness of samples	0.33	0.33	0.31	0.32	0.37
Var H`	0.00018	0.00022	0.00025	0.0003	0.00031
t-test* difference DKC 3421 YG to:	4.66	3.7	7.1	6.1	

*degree of freedom for t-test over 120 (>1.96); ns - not significant.

Table 4. Ecological analysis of ground beetle diversity in Głuchów in 2008-2010

Diversity parameters	Ref. 1 Prot.	Ref. 2 Prot.	DKC 3420 Prot.	DKC 3420 Non-Prot.	DKC 3421 YG
2008					
No. of individuals (N)	10702	11082	9999	10009	11190
No. of species (S)	44	48	45	45	44
Shannon Index (H`)	1.28	1.33	1.3	1.35	1.31
H/log(N) evenness of samples	0.34	0.34	0.34	0.35	0.35
Var H`	0.00016	0.00015	0.00018	0.00018	0.00015
t-test* difference DKC 3421 YG to:	ns	ns	ns	2.38	
2009					
No. of individuals (N)	6367	6776	7167	7372	7096
No. of species (S)	39	34	36	35	37
Shannon Index (H`)	0.98	0.83	0.91	0.82	0.84
H/log(N) evenness of samples	0.27	0.23	0.26	0.23	0.23
Var H`	0.00041	0.00035	0.00034	0.00031	0.00033
t-test* difference DKC 3421 YG to:	5.46	ns	3.05	ns	
2010					
No. of individuals (N)	7846	6780	8340	8005	8282
No. of species (S)	37	27	34	35	33
Shannon Index (H`)	0.8	0.82	0.75	0.83	0.69
H/log(N) evenness of samples	0.22	0.25	0.21	0.23	0.20
Var H`	0.00027	0.00029	0.00024	0.00028	0.00024
t-test* difference DKC 3421 YG to:	4.88	5.6	3.05	6.3	

*Degree of freedom for t-test over 120 (>1.96); ns - not significant.

Despite indices H` and particularly E being low in all sites, the studied variety compared to other varieties was lowest. Nevertheless, the number of caught individuals (6780) and species (27) was lowest in the protected variety Ref. 2. The structures of caught species were very similar for isogenic varieties. However, the evenness index was low on all treatments. This is quite typical for cereal cultivation. The small number of species in a peripheral position in the dimension of the 2 axis is a standard attribute of diversity in agricultural landscapes where maize is grown. This phenomenon does not relate to the variety YG only. Peripheral species usually occur on any crop. Throughout the research period and in both locations, species structures in all treatments were strongly prevailing. Calculated minor differences between treatments of DKC variety are also confirmed by short vectors in DCA analysis (Figure 2).

The gradient of ground beetle assemblage structure was determined by applying detrended correspondence analysis (DCA) (Figures 1 and 2). Due to the fact that the results were similar in all study years, we decided to show only selected data, i.e. from 2010 from 2 localities. The data dispersion (DCA analysis, Budziszów 2010) was uniform, with functional clusters common for this experiment. No clear signs of differentiation between treatments were found. In this case, strong prevailing influence was noted, but with other species strongly centred. Radial dispersion of very short vectors proves considerable similarities of species structures. The greatest difference is the contrast of Ref. 1 to DKC 3421 YG, despite the fact that the t- test does not show the highest value. This stems in part from the significant quantitative differences (average weight) in the structure of the species. The trend towards the formation of their aggregates was poorly outlined.

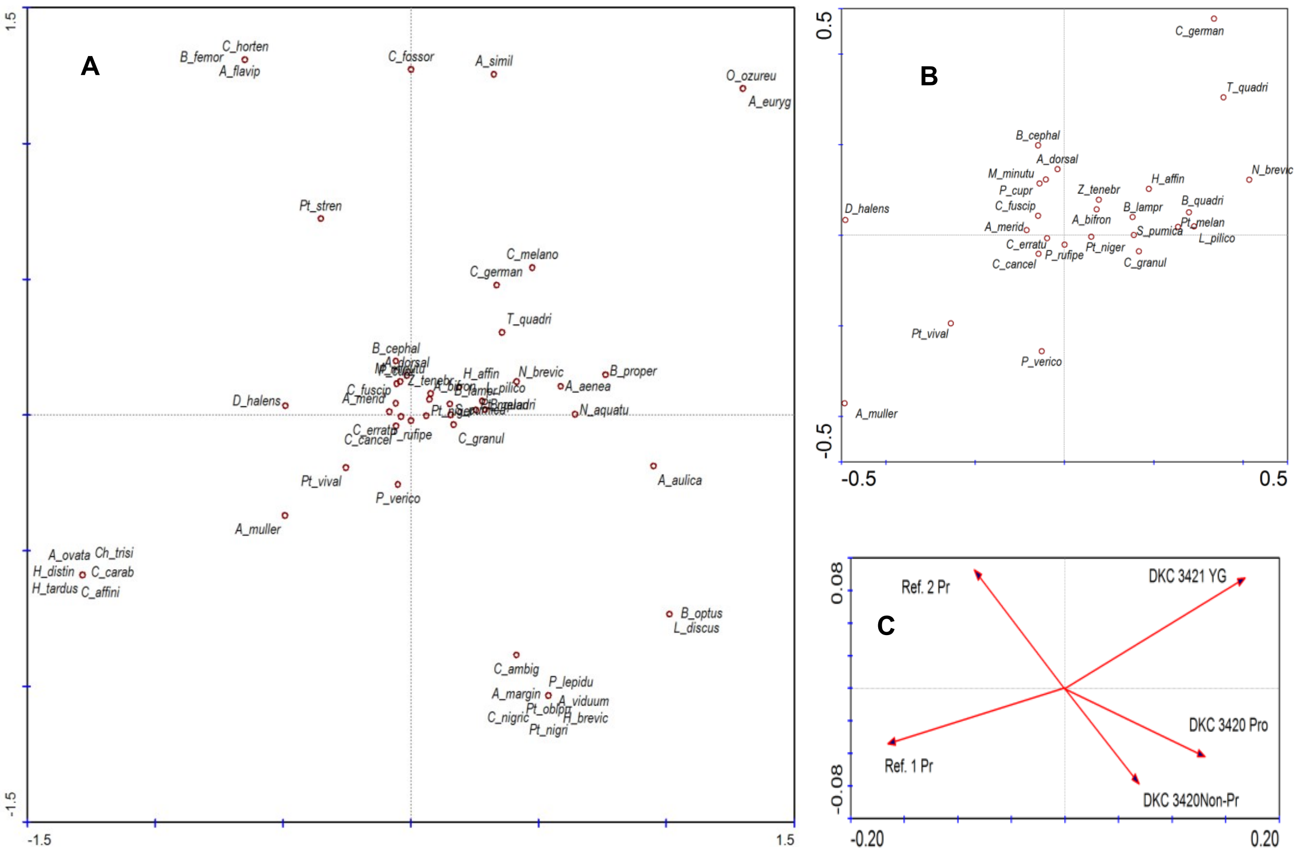

Figure 1. Detrended correspondence analysis (DCA) of ground beetles recorded at Budziszów in 2010 (A: plain view; B: central part - zoom; C: treatment vectors)

In the second locality, in 2010, DCA analysis showed a considerable dispersion of the species, especially along the vertical axis (Figure 2). These are sporadic species, collected in the particular treatments.

Only slight qualitative differences are visible between treatments. Very short vectors are an effect of similarities, but also unbalanced species structure, since more than half of the collected specimens were Pterostichus melanarius. The difference between DKC 3421 YG and Ref. 1 are stronger than in relation to Ref. 2. This is mainly due to the strong influence of quantitative features and was similar for all objects.

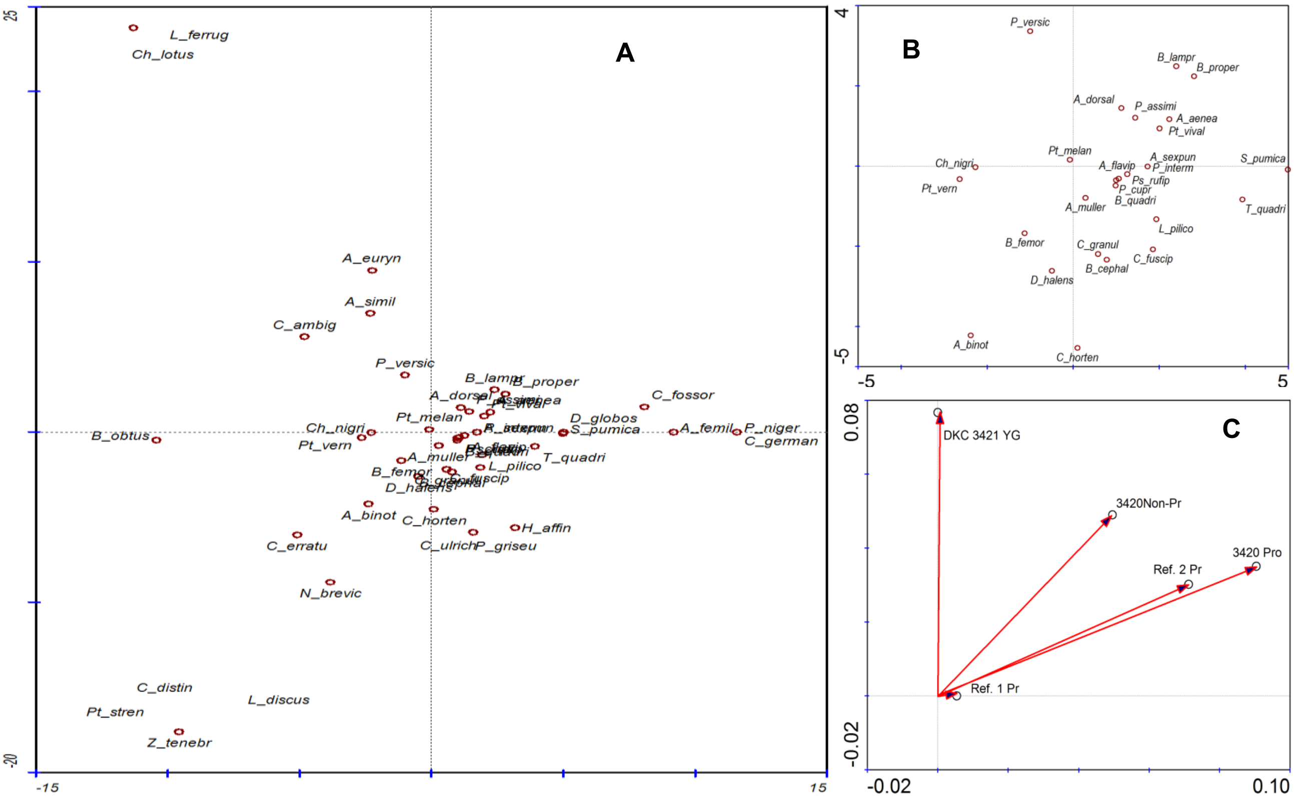

Figure 2. Detrended correspondence analysis (DCA) of ground beetles recorded at Głuchów in 2010 (A: plain view; B: central part - zoom; C: treatment vectors)

Obtained data indicate that the Bt maize strategy had no adverse effect also on the species composition of epigeic ground beetles, which is in agreement with previous investigations of this genetically modified variety (Kocourek et al., 2013; Szekeres et al., 2006; Toschki et al., 2007; Twardowski et al., 2010). Post-market environmental monitoring conducted by Skoková Habuštová et al. (2015), also proved that ground-dwelling ground beetles, as well as other epigeal arthropods (rove beetles and spiders) were not impacted by Bt maize. This comprehensive field study revealed no environmental risks for ground beetles posed by the cultivation of the maize hybrid DKC 3421 YG (event MON 810). In a specific quality approach we found species-rich ground beetle assemblages, with very high individual numbers, especially in one of the studied localities (Budziszów). In both localities the strong domination of only two species was described. Omnivorous *Pseudoophonus rufipes* comprised around 80% of the beetles collected in Budziszów, and together with carnivorous *Pterostichus melanarius*, also more than 80% in second locality. It is obvious that these two species had a significant impact on both quantitative as well as qualitative analysis. All analyses, including ecological and detrended characteristic of beetle assemblage, also did not show any differences between the Bt maize and four other treatments. Other studies conducted in Europe differed in terms of species composition, although *P. rufipes* and *P. melanarius* dominated in many cases, as they are common in this world region and typical for agricultural landscapes (Thiele, 1977).

CONCLUSIONS

In general, we did not find significant differences in the abundance and quality of the total ground beetle assemblages in Bt maize in comparison to conventional varieties in either of the research areas. Some minor differences were detected, but with no clear tendencies related to Bt maize. Our findings rather suggest that crop management practices and/or environmental conditions had the greater impact on ground beetle assemblages and the detected minor differences, than the crop itself (Bt or isoline).

Acknowledgements

This research was realized in the framework of the project "Environmental and economic aspects of permitting cultivation of GM crops in Poland", no. PBZ-MNiSW-06/I/2007, funded by the Polish Ministry of Science and Higher Education.

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